



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

ON THE RELATIVE VIRULENCE OF SENSITIZED AND NON-SENSITIZED TYPHOID BACILLI *

RUSSELL L. CECIL

(From the Medical Clinic of the Presbyterian Hospital, New York City)

In 1910 Garbat and Meyer¹ published the results of an experimental study of a typhoid immune serum produced in rabbits by repeated injections of typhoid bacilli. These investigators found that rabbits, injected intravenously with killed sensitized typhoid bacilli, developed a serum of higher protective power than the rabbits injected with killed, non-sensitized bacilli. Furthermore, they noticed that the animals endured the sensitized better than the non-sensitized bacilli and recovered more quickly. These investigators were concerned chiefly with the relative power of the serum produced by the injection of sensitized and non-sensitized typhoid bacilli; the question of the relative virulence of the bacilli was only casually touched upon. Ficker² has pointed out that the two antigens, as prepared by Garbat and Meyer, are not comparable, since the sensitized bacilli were washed and the non-sensitized unwashed.

Besredka³ found that the sensitized vaccine of typhoid and cholera (killed by heating one hour at 60 C.), when injected intraperitoneally in guinea-pigs, was less toxic than the unsensitized vaccine. Besredka has also shown that subcutaneously the living, sensitized typhoid bacilli are much better tolerated than killed, non-sensitized ones. In view of this fact and the superior immunizing properties of the sensitized vaccine, Besredka has recommended its use in practice instead of the usual vaccine of Wright.

While there seems to be no doubt of this difference in virulence when the bacilli are injected subcutaneously or intraperitoneally, the evidence in the case of intravenous injection is not conclusive. The present experiments were undertaken to settle this point. In view of our recent knowledge of anaphylaxis, it was hardly to be supposed that sensitized typhoid bacilli, injected intravenously, would be less virulent than non-sensitized bacilli. Indeed, the contrary might have been

* Received for publication September 22, 1914.

1. Ztschr. f. exper. Path. u. Therap., 1910, 8, p. 1.

2. Handbuch der Pathogenen Mikroorganismen, 1913, 2, p. 172.

3. Ann. de l'Inst. Pasteur, 1902, 16, p. 918.

expected. My experiments, however, support the observation of Garbat and Meyer, namely, that sensitized typhoid bacilli, when injected intravenously, have less virulence than non-sensitized typhoid bacilli.

The same technic was employed throughout the experiments.

A number of agar slants were inoculated with a stock strain of the bacillus typhosus. After 24 hours' incubation at 37 C. the growth was washed off with normal salt solution, and the clumps were broken up by thorough shaking. In the experiments where killed bacteria were used, the next step was to heat the suspension of bacilli in a water bath for one hour at 60 C. The suspension of bacteria (either killed or living) was now divided into two equal parts. To one part were added 5-10 c.c. of inactivated antityphoid rabbit serum; to the other part, an equal quantity of salt solution. The two suspensions were then placed in the water bath at 37 C. for one hour, and then in the icebox for another hour. The sensitized portion now showed marked agglutination. The suspensions were then centrifuged until both were absolutely clear, the supernatant fluid was pipetted off, and the sediment was washed twice with normal saline solution. Each sediment was next rubbed up with a small quantity of salt solution until perfectly homogeneous and then made up to the desired amount with the same solution. The suspensions were not allowed to stand long; they were almost immediately injected into animals.

The typhoid immune serum was obtained from rabbits which had received a number of intravenous injections of typhoid bacilli, first killed bacilli, then living. The serum was always inactivated by heating for one-half hour at 56 C.

Before proceeding to the experiments proper, it seemed desirable to determine the protective power of the antityphoid serum which was to be used. Increasing amounts of a twenty-four-hour broth culture of the bacillus typhosus (homologous strain) were injected intraperitoneally in white mice, while an equal number of mice received corresponding amounts of culture freshly mixed with typhoid immune serum (agglutinating titer = 1-4,000).

Mice Receivin B. Typhosus		Mice Receiving B. Typhosus + 0.2 c.c. of Typhoid Immune Serum	
1. Broth culture 0.5	c.c. Died.	1. Cultures 0.5	c.c. Died.
2. Broth culture 0.1	c.c. Died.	2. Cultures 0.1	c.c.
3. Broth culture 0.01	c.c. Died.	3. Cultures 0.01	c.c.
4. Broth culture 0.001	c.c. Died.	4. Cultures 0.001	c.c.
5. Broth culture 0.0001	c.c.	5. Cultures 0.0001	c.c.
6. Broth culture 0.00001	c.c.	6. Cultures 0.00001	c.c.

It will be seen from the protocol that the serum protected in mice against 100 times the lethal dose of typhoid bacilli.

Experiment 1.—To determine the relative virulence of living, sensitized and non-sensitized typhoid bacilli, Experiment 1 was made.

Suspensions were made up so that 1 c.c. equaled one-half slant agar of the bacillus typhosus. The results with rabbits injected with sensitized typhoid bacilli were as follows:

- Rabbit 1, 1,420 gm. 0.1 slant, intravenously.
- Rabbit 2, 1,400 gm. 0.25 slant, intravenously.
- Rabbit 3, 1,380 gm. 0.5 slant, intravenously.
- Rabbit 4, 1,350 gm. 0.75 slant, intravenously.
- Rabbit 5, 1,320 gm. 1.0 slant, intravenously.

Rabbit 6, 1,260 gm. 1.5 slants, intravenously.

Rabbit 7, 1,220 gm. 2.0 slants, intravenously.

Rabbit 8, 1,220 gm. 2.5 slants, intravenously. Died night after injection.

Rabbit 4 died of coryza six days after injection. Culture from heart's blood gave pure growth of an influenza-like bacillus.

Rabbit 7 died ten days after injection. Culture from heart's blood gave pure growth of streptococcus.

Culture from the heart's blood of Rabbit 8 gave pure growth of *B. typhosus*.

The results with the rabbits injected with non-sensitized typhoid bacilli are as follows:

Rabbit 1, 1,460 gm. 0.1 slant, intravenously.

Rabbit 2, 1,420 gm. 0.25 slant, intravenously.

Rabbit 3, 1,400 gm. 0.5 slant, intravenously.

Rabbit 4, 1,320 gm. 0.75 slant, intravenously. Died night after injection.

Rabbit 5, 1,240 gm. 1.0 slant, intravenously. Died night after injection.

Rabbit 6, 1,240 gm. 1.5 slant, intravenously. Sick, but recovered.

Rabbit 7, 1,160 gm. 2.0 slant, intravenously. Died day after injection.

Rabbit 8, 1,150 gm. 2.5 slant, intravenously. Died night after injection.

Cultures from heart's blood of Rabbits 4, 5, 7, and 8 gave growths of *B. typhosus*.

The results with the guinea-pigs injected with sensitized bacilli are as follows:

Guinea-Pig 1, 285 gm. 0.01 slant, intravenously.

Guinea-Pig 2, 270 gm. 0.02 slant, intravenously.

Guinea-Pig 3, 255 gm. 0.05 slant, intravenously.

Guinea-Pig 4, 250 gm. 0.1 slant, intravenously.

Guinea-Pig 5, 232 gm. 0.2 slant, intravenously.

Guinea-Pig 6, 260 gm. 0.4 slant, intravenously.

Guinea-Pig 7, 240 gm. 0.8 slant, intravenously. Died night after injection.

Guinea-Pig 8, 245 gm. 1.2 slant, intravenously. Died night after injection.

Guinea-Pig 9, 230 gm. 1.6 slant, intravenously. Died night after injection.

The results with the guinea-pigs injected with non-sensitized bacilli are as follows:

Guinea-pig 1, 272 gm. 0.01 slant, intravenously.

Guinea-pig 2, 262 gm. 0.02 slant, intravenously.

Guinea-pig 3, 237 gm. 0.05 slant, intravenously.

Guinea-pig 4, 247 gm. 0.1 slant, intravenously.

Guinea-pig 5, 220 gm. 0.2 slant, intravenously. Died day after injection.

Guinea-pig 6, 280 gm. 0.4 slant, intravenously. Died night after injection.

Guinea-pig 7, 272 gm. 0.8 slant, intravenously. Died 4 days after injection.

Guinea-pig 8, 255 gm. 1.2 slant, intravenously. Ill but recovered.

Guinea-pig 9, 240 gm. 1.6 slant, intravenously. Died night after injection.

Cultures from hearts' blood of all the guinea-pigs that died gave *B. typhosus*.

It will be seen from these experiments that the sensitized vaccine in both rabbits and guinea-pigs, when injected intravenously, is about one third as virulent as the non-sensitized. Two of the rabbits in the sensitized series died from other infections a number of days after the experiment. As typhoid bacilli were not recovered from their blood, there is no reason to suppose that the deaths were due directly to the sensitized virus.

Experiment 2.—To determine the relative virulence of killed, sensitized and non-sensitized typhoid bacilli, Experiment 2 was made.

The results with the rabbits injected with sensitized bacilli are as follows:

Rabbit 1, 1,400 gm. 0.1 slant, intravenously.
Rabbit 2, 1,540 gm. 0.25 slant, intravenously.
Rabbit 3, 1,600 gm. 0.5 slant, intravenously.
Rabbit 4, 1,500 gm. 1.0 slant, intravenously.
Rabbit 5, 1,410 gm. 1.5 slant, intravenously.
Rabbit 6, 1,150 gm. 2.0 slant, intravenously. Died 2 hours after injection.

The results with the rabbits injected with non-sensitized bacilli are as follows:

Rabbit 1, 1,350 gm. 0.1 slant, intravenously.
Rabbit 2, 1,500 gm. 0.25 slant, intravenously.
Rabbit 3, 1,620 gm. 0.5 slant, intravenously.
Rabbit 4, 1,530 gm. 1.0 slant, intravenously. Died 36 hours after injection.
Rabbit 5, 1,300 gm. 1.5 slant, intravenously. Died few hours after injection.
Rabbit 6, 1,160 gm. 2.0 slant, intravenously. Died day after injection.
Cultures from hearts' blood of rabbits that died were sterile.

The results with the guinea-pigs injected with sensitized bacilli are as follows:

Guinea-pig 1, 260 gm. 0.2 slant, intravenously.
Guinea-pig 2, 247 gm. 0.4 slant, intravenously.
Guinea-pig 3, 245 gm. 0.8 slant, intravenously.
Guinea-pig 4, 225 gm. 1.2 slant, intravenously.
Guinea-pig 5, 215 gm. 1.4 slant, intravenously.
Guinea-pig 6, 233 gm. 1.8 slant, intravenously. Died night after injection.

The results with guinea-pigs injected with non-sensitized bacilli are as follows:

Guinea-pig 1, 270 gm. 0.2 slant, intravenously.
Guinea-pig 2, 245 gm. 0.4 slant, intravenously.
Guinea-pig 3, 240 gm. 0.8 slant, intravenously. Died night after injection.
Guinea-pig 4, 225 gm. 1.2 slant, intravenously. Died night after injection.
Guinea-pig 5, 220 gm. 1.4 slant, intravenously. Ill but recovered.
Guinea-pig 6, 233 gm. 1.8 slant, intravenously. Died night after injection.

Cultures from hearts' blood of guinea-pigs that died were sterile, except one that showed a hay bacillus, apparently a contamination.

These two experiments show that, in the case of typhoid bacilli killed by heating for one hour at 60 C., the sensitized virus, when injected intravenously, is less toxic than the non-sensitized in both rabbits and guinea-pigs. The lethal dose of the sensitized bacteria is two or three times larger than that of the non-sensitized.

It is not at once evident why there should be this difference in virulence between sensitized and non-sensitized bacteria. Besredka⁴ has studied the local reactions produced by the subcutaneous injection of typhoid bacilli, and has found that phagocytosis is much more active with sensitized than with non-sensitized bacteria. It is possible that a similar difference as regards phagocytosis is present when the bacteria

4. Virchows Arch. f. path. Anat., 1913, 213, p. 244.

are injected intravenously and that bacteriolysis is also hastened when the bacilli are first subjected to the action of an immune serum.

CONCLUSIONS

Sensitized, living typhoid bacilli, when injected intravenously in rabbits and guinea-pigs, are less virulent than non-sensitized, living typhoid bacilli.

Sensitized typhoid bacilli, killed by heat, are in a similar way less virulent than non-sensitized, killed typhoid bacilli.

The most probable explanation for this difference is that sensitized typhoid bacilli undergo phagocytosis and bacteriolysis more rapidly than the non-sensitized bacilli.